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Abbreviations: GWAS, genome-wide association studies; SNP, single nucleotide polymorphism.

Abstract

Psoriasis is a chronic inflammatory disease with a complex genetic architecture. To date, the psoriasis heritability is only partially explained. However, there is increasing evidence that the missing heritability in psoriasis could be explained by multiple genetic variants of low effect size from common genetic pathways. The objective of the present study was to identify new genetic variation associated with psoriasis risk at the pathway level. We genotyped 598,258 SNPs in a discovery cohort of 2,281 case-control individuals from Spain. We performed a genome-wide pathway analysis using 1,053 reference biological pathways. A total of 14 genetic pathways (P_{FDR}≤2.55e-2) were found to be significantly associated with psoriasis risk. Using an independent validation cohort of 7,353 individuals from the UK, a total of 6 genetic pathways were significantly replicated (P_{FDR}≤3.46e-2). We found genetic pathways that had not been previously associated with psoriasis risk like retinol metabolism ($P_{combined}$=1.84e-4), the transport of inorganic ions and amino acids ($P_{combined}$=1.57e-7) and post-translational protein modification ($P_{combined}$=1.57e-7). In the latter pathway, *MGAT5* showed a strong network centrality, and its association with psoriasis risk was further validated in an additional case-control cohort of 3,429 individuals (P<0.05). These findings provide insights into the biological mechanisms associated with psoriasis susceptibility.

Introduction

Psoriasis is a common chronic inflammatory disease of the skin that affects approximately 2% of the worldwide population (Nestle *et al.*, 2009). In psoriasis, immune cells infiltrate the skin leading to an increased proliferation of keratinocytes (Ferenczi *et al.*, 2000; Gudjonsson and Elder, 2007). It is a genetically complex disease with a complex mode of inheritance (Vyse and Todd, 1996). HLA class I gene *HLA-C*0602* haplotype association explains the largest part of the known heritability of psoriasis (Nair *et al.*, 2006; Strange *et al.*, 2010).

Genome-wide association studies (GWAS) have been successful in the characterization of the genetic architecture of many complex human diseases (Manolio, 2010). To date, more than 15 GWAS have been performed using large psoriasis cohorts from Caucasian and Asian populations and have collectively identified more than 50 susceptibility loci for psoriasis (Bowes *et al.*, 2015; Tsoi *et al.*, 2015b; Yin *et al.*, 2015; Zuo *et al.*, 2015). Despite progress in characterizing psoriasis genetic etiology, loci outside the HLA region only explain <25% of the estimated psoriasis heritability (Tsoi *et al.*, 2012; Yin *et al.*, 2014).

Recent research has shown that the missing heritability of complex human diseases can be explained by common genetic variants, rare variants or a combination of genetic, epigenetic and environmental interactions (Gibson, 2012). From these, common genetic variants could explain >60% of the heritability of the most prevalent autoimmune diseases (Golan *et al.*, 2014). Importantly, most of these common genetic variants are characterized by having low effect sizes (Park *et al.*, 2010).

Although GWAS based on single markers have successfully identified disease-susceptibility variants, this strategy is not adequate to identify genetic variants with low effect sizes that are genuinely associated with disease risk (Du *et al.*, 2012). In single-marker GWAS, a large

number of genetic variants are tested for association with a complex trait. In order to avoid false positive results, a stringent genome-wide significant threshold must be used (Johnson *et al.*, 2010). This conservative threshold, however, does not allow the identification of modest effect risk loci, unless extremely large samples sizes of cases and controls are used (Wang *et al.*, 2010). Importantly, single-marker GWAS consider only the individual effect of each SNP and ignore the joint effect of multiple causal genetic variants as well as the biological context where disease genes operate (Zhang *et al.*, 2010).

Functionally related genes have been shown to collectively contribute to disease susceptibility, including those loci that do not reach individually the genome-wide significant threshold (Zhong *et al.*, 2010). Recently, new methods that are able to analyze genetic associations at the pathway level have been developed (Gui *et al.*, 2011). Pathway-based approaches are robust statistical methodologies that integrate genetic and biological knowledge in order to test whether sets of functionally related genes are jointly associated with a complex trait (Ramanan *et al.*, 2012). Therefore, pathway-based methods increase the statistical power of the association analysis by reducing the number of association tests that must be performed and allow a functional interpretation of the results (Wu *et al.*, 2010).

Pathway-based analyses have been recently performed to study the genetic basis of cancer subtypes using either selected candidate pathways, but also at a genome-wide scale (Chen *et al.*, 2014; Koster *et al.*, 2014). Although the genome-wide pathway analysis can have a high computational cost, this approach is able to identify novel genetic pathways associated with disease risk. The identification of new pathways associated with disease risk could increase the probability to develop new therapeutic strategies in complex diseases like psoriasis. To date, however, the genome-wide pathway analysis approach has not been performed in psoriasis.

To gain a better understanding of the genetic risk basis of psoriasis, we have performed a genome-wide pathway analysis on a large multicenter cohort of psoriasis patients. In the present study we have analyzed the association of 1,053 reference biological pathways using 1,263 psoriasis patients and 1,558 controls from Spain. Using an independent cohort of 2,178 cases and 5,175 controls from UK, we have then performed a validation study of the significantly associated pathways in the discovery cohort. With this approach, we have identified genetic pathways that had not been previously associated with psoriasis risk like retinol metabolism, transport of inorganic ions and amino acids and post-translation protein modification. These results provide important insights into the genetic etiology of psoriasis.

Results

Identification of genetic pathways associated with psoriasis risk

In the discovery stage, the genome-wide pathway analysis identified a total of 26 genetic pathways significantly associated with psoriasis risk after multiple test correction (P_{FDR}<0.05, Table S1). The complete results of the genome-wide pathway analysis performed in the discovery study are shown in Table S2.

From the 26 significantly associated pathways, we found that 14 pathways included *IL12B* gene. After *HLA-C*0602*, *IL12B* is one of the strongest known genetic risk factors for psoriasis. In order to confirm that the observed pathway associations were the result of the joint effect of multiple genes and not the result of a single risk locus strongly associated with the disease, we removed *IL12B* from these genetic pathways and tested again for association. After extracting *IL12B*, two genetic pathways -"Inflammatory response" and "Natural killer T

cell"- remained significantly associated with psoriasis risk (P_{FDR}<0.05). Consequently, only these two pathways from the group containing *IL12B* gene were selected for replication. Together with the other 12 pathways, a total of 14 different genetic pathways were finally tested for validation in the UK population. Using this independent case-control cohort we significantly validated the association of 9 genetic pathways with psoriasis risk (P_{FDR}<0.05, Table 1).

Characterization of the genetic pathways associated with psoriasis risk

In order to discard the presence of redundant pathways, we evaluated the level of gene overlap between all associated pathways. From the 9 validated genetic pathways, we found that the "Amino acid transport across the plasma membrane" and "Transport of inorganic ions and amino acids" pathways, as well as the "Asparagine N-linked glycosylation", "Transport to the Golgi and subsequent modification" and "Post-translational protein modification" pathways had a high degree of overlap between them (>95% of shared genes, Figure 1a). Consequently, and in order to avoid redundancy, only the pathway showing the highest level of significance was selected to represent each biological process. The "Transport of inorganic ions and amino acids" ($P_{combined}$=1.57e-7, Figure 1b) and "Post-translational protein modification" ($P_{combined}$=1.57e-7, Figure 1c) pathways were therefore selected from each overlapping pathway group. The "Inflammatory response" ($P_{combined}$=1.06e-12), "Natural killer T cell" ($P_{combined}$=1.06e-12), "DNA repair" ($P_{combined}$=1.10e-9) and "Retinol metabolism" ($P_{combined}$=1.84e-4) pathways did not show a significant degree of overlap and were therefore considered as independent biological processes.

Within the final group of 6 genetic pathways associated with disease risk and representing independent biological processes, we analyzed the association between each particular gene

and psoriasis risk (Table 2). We found 37 small-effect genes that were nominally associated with psoriasis risk both in the discovery and replication cohorts (P≤1.29e-2, Table 3). The complete list of genetic associations obtained from each genetic pathway is shown in Table S3. The linkage disequilibrium pattern between the SNPs mapping to each genetic pathway associated with psoriasis risk is shown in Figure S1.

Functional-based networks associated with psoriasis risk

In order to understand the relevance of each particular gene within the genetic pathway associated with psoriasis risk, we used biological knowledge to build the associated functional-based network (Figure 2). Using known or predicted functional associations between the pathway genes, functional-based networks are a powerful approach to represent and analyze the topological structure of a biologic pathway.

In order to characterize the network properties of the resulting functional-based networks, we determined the betweenness centrality (BC) and degree centrality (DC) statistics (Table S4). These two measures are useful to identify those network elements (genes in this case) that are likely to be more influential in the structure of the network. BC and DC have been widely used to identify the genes that are more likely to be essential for pathway functionality (Hahn and Kern, 2005; Joy *et al.*, 2005; Vallabhajosyula *et al.*, 2009). We found that *SLC7A11* from the "Transport of inorganic ions and amino acids" pathway and *MGAT5* from the "Post-translational protein modification" pathway had markedly high BC values (BC≥0.1). From these, *MGAT5* gene showed also a much stronger DC value than *SLC7A11* (DC_{MGAT5}=19, $DC_{SLC7A11}$=3).

Given the strong network centrality properties found for *MGAT5* gene in the "Post-translational protein modification" pathway, we decided to further test the association of this

key gene with psoriasis risk in an independent case-control cohort. Using this additional replication cohort, we significantly validated the association of *MGAT5* gene with psoriasis risk (*P*=1.3e-2, OR (95% CI)=0.85 (0.74-0.96)).

Functional analysis of *MGAT5* variation

MGAT5 encodes for a key enzyme in the *N*-glycosylation pathway, a post-translational process that is directly implicated in T cell activation and differentiation (Demetriou *et al.*, 2001). In order to assess the functional role of *MGAT5* in psoriasis pathogenesis, we evaluated the association between genetic variation at *MGAT5* gene and the levels of T cell surface glycosylation. Flow cytometry analysis of *in vitro* activated CD4+ and CD8+ T cells obtained from 27 psoriasis patients showed an increase in N-glycosylation levels in patients carrying 1 or 2 copies of the protective allele (G) compared to homozygous individuals for the risk allele (A) (Figure 3). The increased glycosylation levels in individuals carrying at least one copy of (G) allele was observed both in activated CD8+ and CD4+ T cells. In CD4+ T lymphocytes, the glycosylation level was significantly higher in GG homozygotes compared to AA homozygotes (*P*=0.01, Figure 3).

Discussion

Genome-wide association analyses have successfully identified more than 50 loci associated with psoriasis susceptibility. To date, however, the genetic basis of psoriasis is still not completely understood. In the present study we have performed a genome-wide pathway analysis of psoriasis genetic risk. Using a discovery cohort from Spain and an independent cohort from UK, we have identified and validated the association of 6 genetic pathways with

psoriasis susceptibility. Importantly, these validated pathways include biological processes like retinol metabolism, transport of inorganic ions and amino acids and post-translational protein modification that had not been previously associated with psoriasis risk at the genetic level. Additionally, analyzing the network properties of these validated pathways we have found that *MGAT5* gene has a strong centrality in the post-translational protein modification pathway. Using an additional independent case-control cohort from Spain, we have further replicated the association of *MGAT5* with psoriasis risk. Taken together, these findings contribute to a better understanding of the genetic risk basis of psoriasis and provide important insights into the biological mechanisms associated with the disease pathogenesis.

Retinol has been demonstrated to inhibit inflammatory processes in dermatological diseases (Balato *et al.*, 2013). In particular, retinol inhibits the regulatory activity of the nuclear factor kappa B (NFKB) in the skin (Austenaa *et al.*, 2004). NFKB is an established transcriptional factor that regulates multiple proinflammatory genes that are key in psoriasis pathogenesis like tumor necrosis factor and interleukin-17 (Goldminz *et al.*, 2013). The NFKB signaling pathway has also been associated to the regulation of the proliferation of epidermal keratinocytes (Tsuruta, 2009). These findings are consistent with the elevated levels of NFKB that have been found in lesional and non-lesional psoriatic skin samples compared to non-psoriatic skin (Lizzul *et al.*, 2005). Therefore, genetic variation in the retinol metabolism pathway could reduce the retinol production leading to a weakened NFKB signaling and, consequently, promoting both inflammatory and proliferative hallmarks of psoriasis.

Psoriasis risk was also associated with the genetic pathway implicated in the transport of both inorganic ions and amino acids. An increased transport of inorganic ions in CD4+ helper T cells has been shown to contribute to autoimmune and inflammatory diseases (Lang *et al.*, 2014). In particular, the intracellular transport of calcium is crucial for controlling the

expression of proinflammatory genes in immune cells (Khananshvili, 2013; Vig and Kinet, 2009). Accordingly, the transport of inorganic ions and amino acids pathway associated with psoriasis risk includes the *SLC8A1* gene, which modulates the cytoplasmic calcium concentration (Clapham, 2007). The transport of amino acids into T cells is essential to maintain the increased production of proinflammatory cytokines in activated human T cells (Hayashi *et al.*, 2013). Importantly, the expression of amino acid transporters has been found to be differentially regulated in psoriatic inflammatory processes (Jaeger *et al.*, 2008). These results therefore suggest that genetic variation in the transport of amino acids and inorganic ions pathway could increase the risk to develop psoriasis by modulating T cell functionality.

The post-translational protein modification pathway is responsible for the N-linked glycosylation of the asparagine residues in the HLA molecules (Rudd *et al.*, 2001). This post-translational modification pathway has been found to be necessary for the immune system tolerance to self antigens (Ryan and Cobb, 2012). Previous studies have found that a deficient or aberrant asparagine glycosilation can induce autoimmune diseases (Green *et al.*, 2007). Also, post-translationally modified autoantigens have been associated with psoriasis (Iversen *et al.*, 2011). In psoriasis patients, the peptide glycosylation activity has been found to be markedly increased in comparison to healthy controls (Damasiewicz-Bodzek and Wielkoszynski, 2012). Furthermore, specific post-translational modifications on glycoproteins expressed on the surface of T lymphocytes have been shown to target these cells to the inflamed skin (Fuhlbrigge *et al.*, 1997). Therefore, genetic variation in the post-translational protein modification pathway could perturb the glycosylation processes that are crucial to maintain the immune system tolerance.

MGAT5 encodes for a key enzyme in the *N*-glycosylation pathway. This pathway has been directly implicated in T cell activation and autoimmunity (Demetriou *et al.*, 2001). Recent

research has found an association between MGAT5 glycosylation activity and multiple sclerosis aetiology both in experimental models and in humans (Grigorian and Demetriou, 2011; Mkhikian *et al.*, 2011). In the present study, we have found that the *MGAT5* is a key gene in the post-translational protein modification pathway associated with psoriasis. Subsequently, we found that genetic variation at *MGAT5* is associated with the level of glycosylation of *in vitro* activated T cells. This result is consistent with previous findings showing that deficiency of MGAT5 glycosylation activity reduces the T-cell activation threshold and, consequently, promotes the triggering of autoimmune diseases (Demetriou *et al.*, 2001). Further studies evaluating the implication of the T cell surface glycosylation in clinically relevant outcomes in psoriasis like skin severity are warranted.

The association of psoriasis risk with the inflammatory response and the natural killer T cell pathways involves more than 10 immune-related genes, including *IL12B*. In a recent pathway analysis study using association results of a meta-analysis for psoriasis risk (Tsoi *et al.*, 2015a), these two pathways were also found to be associated. These findings, however, were not validated using an independent cohort. Our study, therefore, provides strong confirmation of the implication of these two genetic pathways in the risk of psoriasis. Also, the permutation based approach used in our study allowed to control for the potential bias associated with the presence of strong LD patterns within genes. Our results indicate that the association of these pathways is not only driven by *IL12B* gene, but it is the result of the joint contribution of other small-effect genes in these pathways. One of these genes is *CXCR4*, which encodes for a chemokine receptor from the natural killer T cell pathway (Colantonio *et al.*, 2002). Although *CXCR4* gene has not been previously associated with psoriasis risk in single-marker GWAS, CXCR4 chemokine has been shown to reduce keratinocyte proliferation and, consequently, the expansion of psoriatic plaques by regulating the proliferative cytokine signals that are

activated in psoriatic lesions (Takekoshi *et al.*, 2013). In addition, the inflammatory angiogenesis of psoriatic skin that leads to vascular remodeling has been recently shown to be modulated by CXCR4 chemokine (Zgraggen *et al.*, 2014). Using the pathway analysis we can therefore identify small-effect genes like *CXCR4* that cannot be detected by single-marker GWAS but that are biologically implicated in key processes of the disease pathophysiology.

In the present study, we have also found a significant association between the DNA repair genetic pathway and psoriasis risk. Together with the dysregulation of immune system processes, the epidermal hyperproliferation is another well-known biological process implicated in the psoriasis pathophysiology (Wolf *et al.*, 2012). The application of ultraviolet radiation in psoriasis skin lesions to induce apoptosis in aberrantly proliferating keratinocytes has proved to be a successful treatment for the clearance of plaque psoriasis in approximately 70% of patients (Weatherhead *et al.*, 2011). The ultraviolet radiation induces DNA damage that promotes the transcription of the DNA repair pathway genes (Roos and Kaina, 2006). Consequently, the enzymatic machinery of the pathway repairs the DNA damage and also triggers the cell death by activating the p53 apoptotic signaling (Lavin *et al.*, 2005). Therefore, these results suggest that genetic variation in the DNA repair pathway promote an inefficient activation of the p53 apoptotic signaling that leads to an increased keratinocyte proliferation, as well as an inefficient response to ultraviolet therapy in psoriasis patients.

Although the pathway-based analysis is a powerful approach to identify small-effect genetic variants associated with disease risk, this methodology is not exempt of limitations. Intergenic SNPs across the whole genome that map physically far away from genes were not included in the present study. These genetic variants could be known risk loci (e.g. rs12188300 is associated with psoriasis risk and is located at >20Kb from *IL12B* gene) or may regulate the expression of genes through *cis*- and *trans*-eQTL mechanisms (Gilad *et al.*, 2008). Also, some

SNPs might not be functionally related to the closest genes. With the increasing regulatory information derived from eQTL and epigenomic data (Bernstein *et al.*, 2010; Martens and Stunnenberg, 2013; Raney *et al.*, 2011), intergenic SNPs could be integrated in the pathway-based analysis in the next years.

The complex linkage disequilibrium structure of the *HLA* region together with the strong association with the susceptibility to multiple common diseases, has been shown to generate false positive results in pathway-based methods (Wang *et al.*, 2010). Following recent studies, in the present study we removed the SNPs mapping to this locus in order to perform the present pathway analysis (Chen *et al.*, 2014). As a result, known pathways associated with psoriasis risk which include genes from the *HLA* region, like the NFKB pathway, were not analyzed in the present study. Importantly, however, in this study we have found and validated the association between genetic pathways related to IL12 signaling, an established genetic risk pathway for psoriasis and psoriasis risk. Also, within the associated pathways there are known risk genes for psoriasis (e.g. *REV3L* and *IL4* within the DNA repair and inflammatory response pathways, respectively). Together, these results confirm the accuracy of the present pathway-based approach to identify relevant genetic variation associated with psoriasis risk.

The present genome-wide pathway analysis has two important strengths. First, we used PLINK software to identify genetic pathways associated with psoriasis risk. This pathway analysis method uses genotype data in contrast to the methodologies that are only based on association statistics. An important limitation of these latter methodologies is that they do not account for the linkage disequilibrium between SNPs. This can result in highly biased results and a significant increase in false positive results (Wang *et al.*, 2010). Instead, the pathway analysis approach that we used, although it can be computationally costly, it efficiently

overcomes these biases by maintaining the correct linkage disequilibrium patterns between SNPs. Finally, compared to previous pathway-based studies in other complex diseases, we have performed a two-stage pathway analysis in two large cohorts from different populations. Using an independent population, we have validated genetic pathways associated with psoriasis risk.

In conclusion, using a genome-wide pathway analysis approach we have identified to our knowledge previously unreported genetic pathways associated with psoriasis risk. These biological pathways include retinol metabolism, transport of inorganic ions and amino acids and post-translational protein modification. The results of the present study represent an important contribution to the characterization of the genetic risk basis of psoriasis.

Materials and methods

Study population

A total of 1,263 patients with psoriasis and 1,558 controls were recruited for the discovery stage. An independent case-control cohort of 7,353 individuals from UK was used to validate the significantly associated pathways in the discovery cohort. An independent cohort of 1,381 psoriasis patients and 2,048 controls from Spain was used to replicate the association between *MGAT5* gene and psoriasis risk (Supplemental Material)

All the procedures were followed in compliance with the principles of the Declaration of Helsinki and all patients provided written informed consent to participate in this study. The study and the consent procedure were approved by the local Institutional Review Board of each participating center.

DNA extraction and genome-wide genotyping

GWAS genotyping of the 2,821 individuals from the discovery cohort was performed using Illumina Quad610 Beadchips (Illumina, San Diego, California, USA) (Supplemental Material). After the quality control analysis, a final data set of 541,926 SNPs and 1,172 psoriasis patients was available for the pathway-based analysis. The genome-wide genotyping of the psoriasis patients from the validation stage was performed using the Illumina Human660W-Quad (Illumina, San Diego, California, USA) and the healthy controls were genotyped using the Illumina custom Human1.2M-Duo (Illumina, San Diego, California, USA) as it has been previously described (Strange *et al.*, 2010). The final data set used for the replication study included 515,703 SNPs from 2,178 psoriasis patients. The genotyping of the *MGAT5* replication cohort was performed using the Taqman Real-Time PCR platform (Applied Biosystems) (Supplemental Material).

Pathway-based analysis

Gene set definition

Reference biological pathway annotation databases BioCarta (www.biocarta.com), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000) and Reactome (Croft *et al.*, 2014) were used to determine the global pathways (Supplemental Material). The final gene set included in the present study was composed by 215,948 SNPs mapping to 1,053 pathways.

Gene-set association analysis

The statistical association analysis was performed using the PLINK set-based test (Purcell *et al.*, 2007) (Supplemental Material). In order to obtain the global statistical significance of

each validated pathway, we combined the empirical P-values resulting from the discovery and replication stages using the Fisher's method (Kugler *et al.*, 2010). We tested the association of 1,053 pathways with psoriasis risk. The False Discovery Rate (FDR) method (Hochberg and Benjamini, 1990) was used to account for multiple testing.

Sensitivity analysis by removing the HLA and IL12B loci

In pathway-based analysis, the presence of a single marker with very strong effects can lead to false positive associations. In these cases, the joint contribution of the pathway genes to disease risk is masked and not adequately evaluated (Wang *et al.*, 2010). Similar to previous studies, in order to avoid this type of spurious associations, we removed all SNPs mapping to the *HLA* region (Megabases 25.6 to 33.3 in chromosome 6) (Chen *et al.*, 2014). In the discovery stage, we found genetic pathways in which the *IL12B* gene was significantly associated with disease risk at a genome-wide scale. *IL12B* is a well-known psoriasis risk gene that shows a large effect on disease susceptibility and, like *HLA* region, could generate false positive results (Cargill *et al.*, 2007; Nair *et al.*, 2008; Zhu *et al.*, 2013). Accordingly, we removed this psoriasis susceptibility locus (from 158,741,791 to 158,757,481 base pairs in chromosome 5) from the significant pathways and we repeated the analysis. We excluded 73 and 58 SNPs from the discovery and replication studies, respectively.

Characterization of the genetic pathways associated with psoriasis risk

Genetic pathways involved in similar biological processes may share genes. In order to identify pathways representing different and independent biological processes, we computed the gene overlap between each pair of genetic pathways associated with psoriasis risk (Supplemental Material).

The statistical significance of the association between pathway genes and psoriasis risk was

determined according to the most significant SNP mapping to each particular gene.

Analysis of the functional-based networks associated with psoriasis risk

The biological knowledge representing the functional association between gene pairs was used to build the functional-based network of each genetic pathway associated with psoriasis risk. In order to identify those genes that are more likely to play a central role in the genetic pathways associated with psoriasis risk, we analyzed the network statistical properties of each functional-based network (Suplementary Material). Using the genes that were nominally associated with psoriasis risk in both discovery and replication stages, we identified the most influential gene according to the highest values of these network statistics.

Functional analysis of *MGAT5* variation

Following the methodology previously described (Chen *et al.*, 2009), we evaluated the association of *MGAT5* psoriasis risk variant with the level of cell surface glycosylation of *in vitro* activated CD4+ and CD8+ T cells isolated from n=27 psoriasis patients (Supplemental Material).

Conflict of interest

The authors state no conflict of interest.

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Tables

PATHWAY	DATABASE	GENES	$SNPS^{D1}$	P^{D}	FDR^{D}	P^{DE}	FDR^{DE}	$SNPS^{R1}$	P^{R}	FDR^{R}	P^{RE}	FDR^{RE}	P^{C}
Inflammatory response *	Biocarta	29	628	<9.99e-8	5.25e-5	1.35e-2	4.71e-2	606	<3.33e-7	5.77e-7	1.53e-2	1.58e-2	1.06e-12
Natural killer T cell *	Biocarta	29	638	<9.99e-8	5.25e-5	1.17e-2	4.71e-2	603	<3.33e-7	5.77e-7	1.59e-2	1.59e-2	1.06e-12
DNA repair *	Reactome	112	2050	1.33e-4	9.36e-3	-	-	1962	<3.33e-7	5.77e-7	-	-	1.10e-9
Amino acid transport across the plasma membrane	Reactome	31	1025	2.00e-4	1.24e-2	-	-	993	4.00e-5	5.63e-5	-	-	1.57e-7
Post-translational protein modification	Reactome	188	5965	2.00e-4	1.24e-2	-	-	5725	4.00e-5	5.63e-5	-	-	1.57e-7
Transport to the Golgi and subsequent modification	Reactome	33	1557	3.33e-4	1.95e-2	-	-	1516	3.20e-3	4.38e-3	-	-	1.57e-5
Asparagine N-linked glycosylation	Reactome	81	2760	4.00e-4	2.11e-2	-	-	2639	8.67e-3	1.13e-2	-	-	4.72e-5
Transport of inorganic ions and amino acids	Reactome	94	4010	4.00e-4	2.11e-2	-	-	3872	2.00e-5	3.25e-5	-	-	1.57e-7
Retinol metabolism	KEGG	64	1512	5.33e-4	2.55e-2	-	-	1406	2.79e-2	3.46e-2	-	-	1.84e-4

Table 1. Pathways associated with psoriasis risk and validated in the replication stage.

Abbreviations: C, combined; D, discovery cohort; E, exclusion *IL12B* gene; FDR, False Discovery Rate; P, empirical set-based P-value; R, replication cohort.

[1] Number of Single-Nucleotide Polymorphisms mapping to a particular pathway.

* Increased permutations to refine the P-value (n=10,000,000).

PATHWAY[1]	DATABASE	SNP^{D}	COORD	A1	A2	OR^{D}	P^{D}	GENE^{D}	SNP^{R}	COORD	A1	A2	OR^{R}	P^{R}	GENE^{R}
Inflammatory response	Biocarta	rs20541	5:131995964	A	G	0.72	4.18e-5	*IL13,IL4*	rs2965012	1:218786549	A	C	0.83	7.56e-4	*TGFB2*
		rs11739623	5:131864152	A	G	1.21	1.79e-3	*IL5*	rs2243123	3:159709651	G	A	1.14	1.06e-3	*IL12A*
		rs2799083	1:218581617	G	A	1.22	2.82e-3	*TGFB2*	rs25890	5:131437562	G	A	0.88	1.09e-3	*CSF2*
		rs2366408	3:159696099	A	C	1.19	3.35e-3	*IL12A*	rs20541	5:131995964	A	G	0.86	2.41e-3	*IL13,IL4*
		rs2069837	7:22768027	G	A	1.33	3.93e-3	*IL6*	rs4963517	12:6947800	A	G	0.90	2.94e-3	*CD4*
Natural killer T cell	Biocarta	rs20541	5:131995964	A	G	0.72	4.18e-5	*IL4*	rs4297265	1:67852335	G	A	0.83	4.01e-7	*IL12RB2*
		rs11739623	5:131864152	A	G	1.21	1.79e-3	*IL5*	rs749873	2:136817088	G	A	0.84	2.61e-5	*CXCR4*
		rs2799083	1:218581617	G	A	1.22	2.82e-3	*TGFB2*	rs2965012	1:218786549	A	C	0.83	7.56e-4	*TGFB2*
		rs2114808	2:137249556	G	A	0.81	3.09e-3	*CXCR4*	rs2243123	3:159709651	G	A	1.14	1.06e-3	*IL12A*
		rs2366408	3:159696099	A	C	1.19	3.35e-3	*IL12A*	rs25890	5:131437562	G	A	0.88	1.09e-3	*CSF2*
Retinol metabolism	KEGG	rs2173201	4:100250970	A	C	0.77	5.82e-5	*ADH1C,ADH1B*	rs7188923	16:81336356	A	G	0.89	1.81e-3	*BCMO1*
		rs4148295	4:70475866	C	A	1.23	3.41e-4	*UGT2A1*	rs10882144	10:94852448	A	G	0.87	2.55e-3	*CYP26A1*
		rs17614939	4:70360229	G	A	0.78	5.21e-4	*UGT2B4*	rs4319546	12:57346828	A	G	0.89	4.96e-3	*RDH16*
		rs2279345	19:41515702	A	G	0.84	2.44e-3	*CYP2B6*	rs4405788	2:72235688	A	G	0.90	5.48e-3	*CYP26B1*
		rs17864686	2:234591339	A	G	1.25	3.37e-3	*UGT1A8*	rs11670760	19:41336795	G	A	1.12	5.73e-3	*CYP2A6*
DNA repair	Reactome	rs240956	6:111616051	A	C	1.46	3.16e-6	*REV3L*	rs458017	6:111696091	G	A	1.65	1.40e-13	*REV3L*
		rs20541	5:131995964	A	G	0.72	4.18e-5	*RAD50*	rs2240116	9:35094373	A	G	1.36	5.22e-4	*FANCG*
		rs2213178	8:48816716	A	G	1.29	6.11e-5	*PRKDC*	rs7099120	10:131015367	A	G	1.15	9.51e-4	*MGMT*
		rs2985689	14:50098031	C	A	1.28	1.66e-3	*POLE2*	rs3783819	14:61316264	A	G	0.89	1.13e-3	*MNAT1*
		rs1887181	10:131594850	G	A	1.46	1.86e-3	*MGMT*	rs11693731	2:58887650	A	G	0.89	1.13e-3	*FANCL*
Post-translational protein modification	Reactome	rs1007108	1:26104973	A	G	1.43	2.74e-6	*MAN1C1*	rs9886302	7:70751484	A	G	0.81	7.29e-6	*WBSCR17*
		rs10865331	2:62551472	A	G	1.25	7.88e-5	*B3GNT2*	rs7220464	17:7210836	A	C	0.85	2.09e-5	*EIF5A*
		rs3791312	2:135183045	G	A	0.71	8.04e-5	*MGAT5*	rs4528932	3:118941441	A	G	1.17	5.28e-5	*B4GALT4*
		rs1495086	8:15378013	A	G	0.78	1.02e-4	*TUSC3*	rs7780461	7:151641016	A	G	1.24	8.26e-5	*GALNTL5*
		rs977905	3:5882683	G	A	1.24	1.69e-4	*EDEM1*	rs12262718	10:17343706	A	G	1.31	8.68e-5	*ST8SIA6*
Transport of inorganic ions and amino acids	Reactome	rs12661704	6:111560890	A	G	1.60	2.34e-7	*SLC16A10*	rs12661704	6:111560890	A	G	1.42	2.38e-9	*SLC16A10*
		rs10205402	2:40710953	A	G	0.77	8.78e-6	*SLC8A1*	rs2385844	2:220839453	G	A	0.85	9.23e-6	*SLC4A3*
		rs532237	20:48467560	G	C	1.31	1.09e-4	*SLC9A8*	rs6012750	20:48430680	A	G	0.86	6.81e-5	*SLC9A8*
		rs538385	13:30229665	G	A	0.82	6.91e-4	*SLC7A1*	rs1874361	1:205908186	A	C	1.15	1.63e-4	*SLC26A9*
		rs17050441	4:139402774	G	A	1.27	1.14e-3	*SLC7A11*	rs11668878	19:47268373	A	C	1.27	1.65e-4	*SLC1A5*

Table 2. Association results of the top five genes involved in each pathway associated with psoriasis risk.

Abbreviations: A1, minor allele; A2, major allele; COORD, SNP coordinates in build GRCh37/hg19; D, discovery cohort; OR, odds ratio; P, P-value; R, replication cohort.

[1] The detailed description of the "Inflammatory response" and "Natural killer T cell" pathways corresponds to the association results after excluding the *IL12B* gene from the genome-wide pathway analysis.

PATHWAY[1]	DATABASE	GENE[2]	P^D	P^R
Inflammatory response	Biocarta	*IL12A*	3.35e-3	1.06e-3
		IL12B	3.02e-10	1.69e-18
		IL13	4.18e-5	2.41e-3
		IL4	4.18e-5	2.41e-3
		TGFB2	2.82e-3	7.56e-4
Natural killer T cell	Biocarta	*CXCR4*	3.09e-3	2.61e-5
		IL12A	3.35e-3	1.06e-3
		IL12B	3.02e-10	1.69e-18
		IL4	4.18e-5	2.41e-3
		IL4R	7.35e-3	1.37e-3
		TGFB2	2.82e-3	7.56e-4
Retinol metabolism	KEGG	*ADH1B*	5.82e-5	1.21e-2
		UGT2B4	5.21e-4	6.13e-3
		RPE65	5.10e-3	7.34e-3
DNA repair	Reactome	*FANCL*	3.22e-3	1.13e-3
		MGMT	1.86e-3	9.51e-4
		RAD50	4.18e-5	2.41e-3
		REV3L	3.16e-6	1.40e-13
		RFC3	2.14e-3	1.94e-3
Transport of inorganic ions and amino acids	Reactome	*SLC16A10*	2.34e-7	2.38e-9
		SLC1A4	2.04e-3	7.44e-3
		SLC38A1	1.34e-3	9.44e-3
		SLC43A2	1.29e-2	1.15e-2
		SLC7A1	6.91e-4	6.62e-3
		SLC7A11	1.14e-3	6.22e-3
		SLC7A7	5.09e-3	2.77e-3
		SLC8A1	8.75e-6	1.30e-3
		SLC9A8	1.09e-4	6.81e-5
		SLC9A9	4.37e-3	3.22e-3
Post-translational protein modification	Reactome	*ALG10*	3.89e-3	4.15e-3
		B3GNT2	7.88e-5	1.01e-3
		EDEM1	1.69e-4	5.81e-4
		EIF5A	3.42e-4	2.09e-5
		FUT8	5.30e-3	1.87e-4
		GALNT1	5.22e-4	9.72e-4
		MAN1A1	2.82e-4	1.04e-2
		MAN2A1	7.29e-3	3.53e-3
		MGAT5	8.04e-5	9.34e-3
		SEMA6D	2.06e-3	1.46e-3
		ST8SIA6	8.17e-3	8.68e-5
		TUSC3	1.02e-4	6.07e-3

Table 3. Genes associated with psoriasis risk in the discovery and replication stages for each validated pathway.

Abbreviations: D, discovery cohort; OR, odds ratio; P, P-value; R, replication cohort.

[1] The detailed description of the "Inflammatory response" and "Natural killer T cell" pathways corresponds to the association results before excluding the *IL12B* gene from the genome-wide pathway analysis.

[2] Genes contained in the genetic pathways that were nominally associated with psoriasis risk in the discovery and replication stages.

Figure Legends

Figure 1. Gene overlap of genetic pathways associated with psoriasis risk. A: Heat-map representing the percentage of genes that are shared between each pathway pair. **B:** Venn diagram of the overlapping pathways representing the transport of inorganic ions and amino acids process as well as the number of genes shared between them. **C:** Venn diagram of the overlapping pathways representing the post-translational protein modification process as well as the number of genes shared between them.

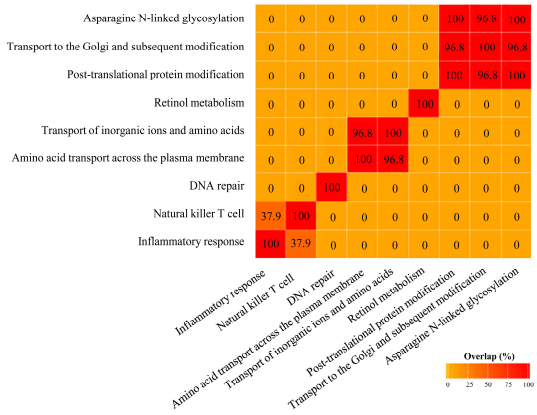
Figure 2. Functional-based network of each genetic pathway associated with psoriasis risk. A: "Inflammatory response". **B:** "Natural killer T cell". **C:** "Retinol metabolism". **D:** "DNA repair". **E: "**Transport of inorganic ions and amino acids". **F: "**Post-translational protein modification". The color of each gene represents the P-value of its association with psoriasis in the negative logarithmic scale, ranging from the lowest significance (green) to the strongest (red). The gene shape represents the association with the disease in neither the discovery nor the replication study (square), only in either the discovery or the replication study (circle) and the association found in both discovery and replication studies (rhombus). The edge width is proportional to the confidence of the functional association between two genes. Disconnected genes are hidden.

Figure 3. N-Glycosylation on activated T lymphocytes according to *MGAT5* genotype. Boxplots of mean fluorescence intensity (MFI) of cell membrane glycosylation of *in vitro* activated CD4+ (left) and CD8+ (right) T cells from psoriasis patients. Patients with one and two copies of the protective (G) allele of *MGAT5* SNP rs3791318, tend to have higher glycosylation levels, thus increasing the threshold for T cell receptor mediated response as well as lowering the threshold for cytotoxic T-lymphocyte-associated antigen-4 mediated arrest of T cell proliferation.

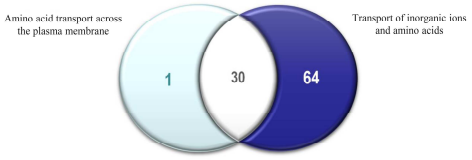
Supplemental Material

The supplementary information is available in the online *Supplemental Material* file.

a)



b)



c)